IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

GORFIEN *et al.*

Appl. No. 09/028,514

Filed: February 23, 1998

For: **Serum-Free Mammalian Cell Culture Medium, and Uses Thereof**

Confirmation No.: 4800

Art Unit: 1651

Examiner: Ware, D.

Atty. Docket: 0942.4110002/RWE/FRC

Brief on Appeal Under 37 C.F.R. § 1.192

Mail Stop Appeal Brief - Patents

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

A Notice of Appeal from the final rejection of claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 was filed on August 25, 2003. Appellants hereby file this Appeal Brief in triplicate, together with the required brief filing fee.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

I. Real Party in Interest (37 C.F.R. § 1.192(c)(1))

The real party in interest in this appeal is Invitrogen Corporation.

II. Related Appeals and Interferences (37 C.F.R. § 1.192(c)(2))

Appellants' undersigned representative is not aware of any appeals or interferences related to the captioned application.

III. Status of Claims (37 C.F.R. § 1.192(c)(3))

The captioned application was filed on February 23, 1998. As originally filed, the application contained a total of 139 claims.

In a preliminary amendment filed on September 24, 1999, claims 38-72, 84-105 and 113-139 were cancelled, claim 106 was amended, and claims 140-153 were added.

In an amendment filed on July 28, 2000, claims 78, 83, 141 and 142 were cancelled, claims 1, 15, 22, 106, 112, 146, 152 and 153 were amended, and claims 154-158 were added.

In an amendment filed on May 7, 2001, claims 152, 153 and 157 were amended, and claims 159 and 160 were added.

In an amendment filed on February 1, 2002, claims 4, 5, 18, 19, 25, 26, 110 and 111 were cancelled, claims 1, 3, 15, 17, 22, 24, 106, 107, 109 and 158 were amended, and claims 161-174 were added.

In an amendment filed on November 26, 2002, claims 9, 10 and 168 were amended.

Claims 1-3, 6-17, 20-24, 27-37, 73-77, 79-82, 106-109, 112, 140 and 143-174 are pending in the application.

Claims 79-82, 106-109, 112, 143-153, 155 and 156 are withdrawn from consideration.

Claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 are now on appeal. A copy of the claims on appeal can be found in the attached Appendix.

IV. Status of Amendments (37 C.F.R. § 1.192(c)(4))

All amendments have been entered. No amendments have been filed subsequent to the issuance of the final Office Action dated February 25, 2003.

V. Summary of Invention (37 C.F.R. § 1.192(c)(5))

In many biological applications that involve culturing mammalian cells, it is advantageous to grow the cells in suspension rather than in monolayers. *See* Specification at page 10, line 23, through page 12, line 8. For example, suspension cultures, which grow in a three-dimensional space, generally produce much higher cell yields than cells grown in monolayer culture. *See* Specification at page 11, lines 1-9. In addition, it is often desirable to grow mammalian cells in media that is free of serum and/or other undefined components that might interfere with the growth of the cells or complicate the isolation of biological products produced by cells in culture. *See* Specification at page 4, line 9, through page 7, line 2. Accordingly, the present invention relates to methods for culturing mammalian cells in suspension in serum-free media.

Independent claim 1 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a serum-free, chemically defined cell culture

medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate. Independent claim 15 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a chemically defined cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, at least one polyanionic or polycationic compound, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts, wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate. Claim 154 depends from claim 15 and specifies that the medium is serum-free. Independent claim 22 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a serum-free, chemically defined cell culture medium obtained by combining a basal medium with at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not

contain dextran sulfate. Claim 27 is directed to the method of claim 22, wherein said basal medium is obtained by combining one or more ingredients selected from the group consisting of ethanolamine, D-glucose, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, one or more calcium salts, one or more iron salts, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts, wherein each ingredient is added in an amount which supports the cultivation of said cell in suspension. Independent claim 158 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a serum-free, non-animal derived cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate. Independent claim 161 is directed to a method of cultivating 293 cells in suspension *in vitro*, comprising: (a) obtaining 293 cells to be cultivated in suspension; and (b) contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension. Claim 162 depends from claim 161 and specifies that the medium further comprises at least one polyanionic or polycationic compound. Claim 140 depends from

claim 1 and specifies that the serum-free cell culture medium is free of animal derived ingredients. Support for claims 1, 15, 22, 27, 140, 154, 158, 161 and 162 can be found throughout the specification, for example, at page 13, line 12, through page 15, line 2.

Claim 165 depends from claim 162 and specifies that the polysulfonated or polysulfated compound is dextran sulfate. Claim 166 depends from claim 165 and specifies that the dextran sulfate has an average molecular weight of about 5,000 Dalton. Claim 174 depends from claim 165 and specifies that the dextran sulfate is present in the medium in an amount effective to substantially prevent clumping (cell aggregation). Support for claims 165, 166 and 174 can be found throughout the specification, for example, at page 13, lines 19-20 and at page 32, lines 22-25.

Claims 2, 16, 23 and 163 depend from claims 1, 15, 22, and 162, respectively, and specify that the polyanionic compound is a polysulfonated compound or a polysulfated compound. Claims 3, 17, 24 and 164 depend from claims 2, 16, 23 and 164, respectively, and specify that the polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan. Support for claims 2, 3, 16, 17, 23, 24, 163 and 164 can be found throughout the specification, for example, at page 13, lines 16-20.

Claims 6 and 167 depend from claims 1 and 162, respectively, and specify that the medium is protein-free. Claims 7 and 8 depend from claim 1 and specify that the medium is a 1X medium formulation and a 10X concentrated medium formulation, respectively. Support for claims 6, 7, 8 and 167 can be found throughout the specification, for example, at page 20, lines 5-6.

Claims 9 and 168 depend from claims 1 and 162, respectively, and specify that the medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, transferrin substitutes, insulin, and insulin substitutes. Support for claims 9 and 168 can be found throughout the specification, for example, at page 30, lines 2-5.

Claims 10, 20, 28, and 169 depend from claims 9, 15, 22 and 168, respectively, and specify that the medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides. Claims 11, 21, 29 and 170 depend from claims 10, 20, 28, and 169, respectively, and specify that the one or more plant peptides are one or more rice peptides or one or more soy peptides. Claims 12 and 171 depend from claims 9 and 168, respectively, and specify that the amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. Claims 13 and 172 depend from claims 9 and 168, respectively, and specify that the vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}. Claims 14 and 173 depend from claims 9 and 168, respectively, and specify that the inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$,

Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts. Support for claims 10-14, 20, 21, 28, 29 and 169-173 can be found throughout the specification, for example, at page 14, lines 1-18.

Independent claim 73 is directed to a method of producing a virus comprising (a) obtaining a mammalian cell to be infected with a virus; (b) contacting said cell with a virus under conditions suitable to promote the infection of said cell by said virus; and (c) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of said virus by said cell. Claim 77 depends from claim 73 and specifies that the virus is an adenovirus, an adeno-associated virus or a retrovirus. Support for claims 73 and 77 can be found throughout the specification, for example, at page 18, lines 14-22.

Claim 30, which depends from claims 1, 15 or 22, and claim 74, which depends from claim 73, specify that the mammalian cell is a mammalian epithelial cell. Claim 31 depends from claim 30 and specifies that the mammalian epithelial cell is selected from the group consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell, a tracheal epithelial cell, a kidney epithelial cell and a retinal epithelial cell. Claims 32 and 75 depend from claims 30 and 73, respectively, and specify that the cell is a human cell. Claim 33 depends from claim 32 and specifies that the human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof. Claims 34 and 76 depend from claims 33 and 75, respectively, and specify that the human cell is a 293 embryonic kidney cell. Claim 35 depends from claim 30 and specifies that the cell is a normal cell. Claim 36 depends from claim 30 and specifies that the cell is an abnormal cell. Claim 37 depends from claim 36 and specifies that the abnormal cell is a transformed cell,

an established cell, or a cell derived from a diseased tissue sample. Support for claims 30-36 and 74-76 can be found throughout the specification, for example, at page 16, line 8, through page 17, line 10.

Independent claim 157 is directed to a method for replacing protein in a mammalian cell culture medium, said method comprising replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. Claim 159 depends from claim 157 and specifies that Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L. Claim 160 depends from claim 159 and specifies that the concentration of Fe^{2+} and/or Fe^{3+} is about 0.0011 g/L and the concentration of Zn^{2+} is about 0.000354 g/L. Support for claims 157, 159 and 160 can be found throughout the specification, for example, at page 40, line 3, through page 41, line 3.

VI. Issue on Appeal (37 C.F.R. § 1.192(c)(6))

The issue on appeal is whether claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 are unpatentable under 35 U.S.C. § 103(a) as being obvious over U.S. Patent No. 4,786,599 to Chessebeuf *et al.* ("Chessebeuf") in view of U.S. Patent No. 5,728,580 to Shuler *et al.* ("Shuler") and U.S. Patent No. 5,712,163 to Parenteau *et al.* ("Parenteau"). *See* Office Action dated February 25, 2003, page 2, lines 11-13.

VII. Grouping of Claims (37 C.F.R. § 1.192(c)(7))

For the purpose of this appeal, the pending claims do not stand or fall together. The claims are grouped as follows:

Group I: Claims 1-3, 6, 7, 9-17, 20-24, 27-32, 35-37, 140, 154 and 158;

Group II: Claim 8;

Group III: Claims 33 and 34;

Group IV: Claims 73-75;

Group V: Claim 76;

Group VI: Claim 77;

Group VII: Claim 157;

Group VIII Claims 159 and 160;

Group IX: Claim 161; and

Group X: Claims 162-174.

VIII. Argument (37 C.F.R. § 1.192(c)(8))

A. Legal Standard for Obviousness

In order to establish a *prima facie* case of obviousness, three requirements must be met. First, all the claim limitations must be taught or suggested by the prior art. *See In re Royka*, 490 F.2d 981, 984-85 (CCPA 1974); *see also In re Glaug*, 283 F.3d 1335, 1341-42 (Fed. Cir. 2002) (finding a claim not obvious because the prior art did not teach "spaced zones of adhesive" as recited in the claim); *In re Rijckaert*, 9 F.3d 1531, 1533 (Fed. Cir. 1993) (finding a claim not obvious because the prior art did not teach all claim limitations). Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *See In re Rouffet*, 149 F.3d 1350, 1357 (Fed. Cir. 1998). Third, there must be a reasonable expectation of success. *See In re Merck & Co.*,

Inc., 800 F.2d 1091 (Fed. Cir. 1986). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in Applicants' disclosure. *See In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

Evidence of a suggestion, teaching, or motivation to combine prior art references may flow, *inter alia*, from the references themselves, the knowledge of one of ordinary skill in the art, or from the nature of the problem to be solved. *See In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999). Although a reference need not expressly teach that the disclosure contained therein should be combined with another, *see Motorola, Inc. v. Interdigital Tech. Corp.*, 121 F.3d 1461, 1472 (Fed. Cir. 1997), the showing of combinability, in whatever form, must nevertheless be "clear and particular." *Dembiczak*, 175 F.3d at 999. "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence.'" *Id.* at 999; *see also In re Kotzab*, 217 F.3d 1365, 1371 (Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.")

B. The Subject Matter of the Claims is Not Obvious Over the Cited References

1. The Claims of Group I (Claims 1-3, 6, 7, 9-17, 20-24, 27-32, 35-37, 140, 154 and 158) are Not Obvious over the Cited References

The claims of group I are directed to methods for cultivating a mammalian cell in suspension *in vitro*. The methods comprise obtaining a mammalian cell to be cultivated in suspension, and contacting the cell with a culture medium (*e.g.*, a serum-free and/or chemically defined cell culture medium) comprising at least one polyanionic or polycationic

compound. The media used in the methods of the group I claims do not contain dextran sulfate. The claims of group I are not obvious over the references relied on in the rejection.

Chessebeuf relates to a serum-free culture medium supplemented with certain fatty acids or their esters in the presence of a "lipophile biopolymer." *See* Chessebeuf at column 2, lines 27-34. Chessebeuf does not teach or suggest a culture medium comprising at least one polyanionic or polycationic compound. Chessebeuf does not teach or suggest culturing a *mammalian* cell in suspension.

Shuler relates to culturing a particular *insect* cell line, BTI-Tn-5B1-4 ("TN5B1-4"), in suspension culture in the presence of dextran sulfate or other sulfated polyanions. *See* Shuler at column 3, lines 34-39. Shuler does not teach or suggest the suspension culture of *mammalian* cells. In fact, Shuler teaches away from the use of sulfated polyanions in the culture of mammalian cells; according to Shuler, such compounds have been found to induce aggregation of *mammalian* cells. For example, Shuler states that "[i]n mammalian cells, the results [with sulfated polyanions] are not clear-cut. For example, heparin induces aggregation of lymphoid cells . . ., and polyanions such as dextran sulfate induce aggregation of lymphocyte cells." Shuler at column 4, lines 55-59 (internal citations omitted). Shuler also notes that:

> Other studies have shown that *sulfated polysaccharides induce aggregation of mammalian cells, indicating that these compounds have no intrinsic capacity to disaggregate cultures*, and in fact, disaggregation is an unexpected result. Thurn and Underhill found that heparin and dextran sulfate induced aggregation in cells of lymphoid origins but not in cell lines of fibroblastic origins. The ability of heparin to induce aggregation is closely related to the binding affinity of heparin to the cell surface. Conditions such as a shift in pH or ionic strength which lower the binding affinity of heparin increase aggregation. Nakashima

> et al. reported that *polyanions such as dextran sulfate induce aggregation of mouse lymphocytes*.

Shuler at column 12, lines 1-13 (emphasis added).

The overall impression conveyed by Shuler is that the ability of sulfated polyanions to prevent cell aggregation is specific for TN5B1-4 insect cells. Shuler suggests that TN5B1-4 cells disaggregate in the presence of sulfated polyanions because of the presence of specific adhesion receptors on the surface of the cells. *See* Shuler at column 12, line 64, through column 13, line 14. Shuler distinguishes TN5B1-4 cells, not only from mammalian cells, but even more particularly from other insect cell lines (*e.g.*, SF21 cells), in terms of their ability to be disaggregated by sulfated polyanions. According to Shuler, "[a]dhesion receptors present on the surface of TN5B1-4, but not SF21 cells *interact specifically* with substrate on lysed membrane fragments causing aggregation of TN5B1-4 cells. Sulfated polyanions competitively bind to the substrate on the membrane fragments thus preventing aggregation of TN5B1-4 cells." Shuler at column 12, line 66, through column 13, line 4 (emphasis added). A person of ordinary skill in the art would therefore not expect that results observed using TN5B1-4 cells would be observed using cell types other than TN5B1-4.

Moreover, as mentioned above, Shuler indicates that sulfated polyanions cause mammalian cells to aggregate. A person of ordinary skill in the art would appreciate that effective cultivation of mammalian cells in suspension requires that the cells do not clump or aggregate. "There is no suggestion to combine . . . if a reference teaches away from its combination with another source." *Tec Air, Inc. v. Denso Manufacturing Michigan Inc.*, 192 F.3d 1353 (Fed. Cir. 1999). Thus, in view of Shuler, a person of ordinary skill in the art would have been discouraged from using sulfated polyanions in mammalian cell culture.

Parenteau relates to chemically defined media that allow the culture of cells in the absence of feeder cells, serum, or "other components which may contribute undefined proteins to the media." *See* Parenteau at column 6, line 66, through column 7, line 2. The media of Parenteau are used to culture attached cells or cells on microcarriers. *See* Parenteau at column 6, lines 9-13 and lines 34-40. Parenteau does not teach or suggest a culture medium comprising at least one polyanionic or polycationic compound. Parenteau does not teach or suggest culturing a mammalian cell in suspension.

There is no suggestion to combine or modify the cited references. In particular, a person of ordinary skill in the art would have not been motivated to include at least one polyanionic or polycationic compound in the culture media of Chessebeuf or Parenteau and/or to adapt the culture media of these references to cultivate cells in suspension. In addition, a person of ordinary skill in the art would not have been motivated to apply teachings for the particular insect cell line used in Shuler to mammalian cells. As discussed above, a person of ordinary skill in the art would have been discouraged from using the culture media of Shuler in conjunction with mammalian cells because Shuler indicates that sulfated polyanions induce aggregation of mammalian cells.

In addition, a person of ordinary skill in the art would not have had a reasonable expectation of success in combining the references. In particular, a person of ordinary skill in the art would have appreciated that the conditions and media used to culture insect cells differ significantly from those used to culture mammalian cells. A person of ordinary skill in the art would not have expected that mammalian cells could have successfully been cultured in the insect culture medium of Shuler. Since there is no suggestion to combine or modify the references relied on to craft the rejection, and since there would have been no

reasonable expectation of success in combining or modifying the cited references, the subject matter of the claims of group I cannot be said to be obvious over the references.

2. The Claim of Group II (Claim 8) is Not Obvious over the Cited References

The claim of group II is directed to methods for cultivating a mammalian cell in suspension *in vitro*. The methods comprise obtaining a mammalian cell to be cultivated in suspension, and contacting the cell with a serum-free, chemically defined culture medium comprising at least one polyanionic or polycationic compound. The media used in the methods of the group II claim do not contain dextran sulfate. The medium used in the methods of the group II claim is a 10X concentrated medium formulation.

The claim of group II depends from claim 1 (which is included within group I). The claim of group II therefore is not obvious over the references relied on in the rejection for at least the same reasons that the claims of group I are not obvious over the references. *See* Section *VIII.B.1*, above. In addition, none of the references teach or suggest a 10X concentrated medium formulation. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since not all elements of the claim of group II are taught or suggested by the references relied on in the rejection, the claim of group II is necessarily not obvious over the references.

3. The Claims of Group III (Claims 33 and 34) are Not Obvious over the Cited References

The claims of group III are directed to methods for cultivating a mammalian cell in suspension *in vitro*. The methods comprise obtaining a mammalian cell to be cultivated in suspension, and contacting the cell with a culture medium (*e.g.*, a serum-free and/or chemically defined cell culture medium) comprising at least one polyanionic or polycationic compound. The media used in the methods of the group III claims do not contain dextran sulfate. The claims of group III specify that the mammalian cell is a human cell, wherein the human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof.

The claims of group III depend from claim 32 (which is included within group I). The claims of group III therefore are not obvious over the references relied on in the rejection for at least the same reasons that the claims of group I are not obvious over the references. *See* Section *VIII.B.1*, above. In addition, none of the references teach or suggest methods comprising culturing 293 embryonic kidney cells, HeLa cervical epithelial cells, PER-C6 retinal cells, or a derivatives thereof. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since the particular human cells specified in the claims of group III are not taught or suggested by the references relied on in the rejection, the claims of group III are necessarily not obvious over the references.

4. The Claims of Group IV (Claims 73-75) are Not Obvious over the Cited References

The claims of group IV are directed to methods for producing a virus. The methods comprise obtaining a mammalian cell to be infected with a virus; contacting the cell with a virus under conditions suitable to promote the infection of the cell by the virus; and cultivating the cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of the virus by the cell.

The methods encompassed by the claims of group IV comprise methods of cultivating a mammalian cell in suspension *in vitro* as defined by claims 1, 15, or 22 (which are included within group I). The claims of group IV therefore are not obvious over the references relied on in the rejection for at least the same reasons that the claims of group I are not obvious over the references. *See* Section *VIII.B.1*, above. In addition, none of the references teach or suggest methods of producing a virus comprising contacting a mammalian cell with a virus. Shuler mentions the infection of TN5B1-4 insect cells with a recombinant E2 strain of *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV). *See* Shuler at column 7, lines 22-32. There is no suggestion in Shuler of infecting mammalian cells with a virus. As mentioned above, Shuler teaches away from the use of mammalian cells in the context of the methods described therein. Accordingly, the claims of group IV are not obvious over the references relied on to craft the rejection.

5. The Claim of Group V (Claim 76) is Not Obvious over the Cited References

The claim of group V is directed to methods for producing a virus. The methods comprise obtaining a mammalian cell to be infected with a virus; contacting the cell with a

virus under conditions suitable to promote the infection of the cell by the virus; and cultivating the cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of the virus by the cell. The claim of group V specifies that the mammalian cell is a human 293 embryonic kidney cell.

The claim of group V depends from claim 75 (which is included within group IV). The methods encompassed by the claim of group V comprise methods of cultivating a mammalian cell in suspension *in vitro* as defined by claims 1, 15, or 22 (which are included within group I). Therefore, the methods encompassed by the claim of group V are not obvious over the references relied on in the rejection for at least the same reasons that the claims of groups I and IV are not obvious over the references. *See* Sections *VIII.B.1* and *4*, above. In addition, none of the references teach or suggest the use of human 293 embryonic kidney cells. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since human 293 embryonic kidney cells are not taught or suggested by the references relied on in the rejection, the claims of group V are necessarily not obvious over the references.

6. The Claim of Group VI (Claim 77) is Not Obvious over the Cited References

The claim of group VI is directed to methods for producing a virus. The methods comprise obtaining a mammalian cell to be infected with a virus; contacting the cell with a virus under conditions suitable to promote the infection of the cell by the virus; and cultivating the cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of the virus by the cell. The claim of group VI specifies that the virus is an adenovirus, an adeno-associated virus or a retrovirus.

The claim of group VI depends from claim 73 (which is included within group IV). The methods encompassed by the claim of group VI comprise methods of cultivating a mammalian cell in suspension *in vitro* as defined by claims 1, 15, or 22 (which are included within group I). Therefore, the methods encompassed by the claim of group VI are not obvious over the references relied on in the rejection for at least the same reasons that the claims of groups I and IV are not obvious over the references. *See* Sections *VIII.B.1* and *4*, above. In addition, none of the references teach or suggest the use of an adenovirus, an adeno-associated virus or a retrovirus. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since neither adenoviruses, adeno-associated viruses nor retroviruses are taught or suggested by the references relied on in the rejection, the claims of group VI are necessarily not obvious over the references.

7. The Claim of Group VII (Claim 157) is Not Obvious over the Cited References

The claim of group VII is directed to methods for replacing protein in a mammalian cell culture medium. The methods comprise replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. The claim of group VII is not obvious over the references relied on in the rejection.

None of the references teach or suggest the replacement of protein in a mammalian cell culture medium. More specifically, none of the references teach or suggest (i) replacing insulin with a Zn^{2+} salt; (ii) replacing transferrin with a Fe^{2+} chelate; and/or (iii) replacing transferrin with a Fe^{3+} chelate. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d

at 984-85. Since not all elements of the claim of group VII are taught or suggested by the references relied on in the rejection, the claim of group VII is not obvious over the references.

8. The Claims of Group VIII (Claims 159 and 160) are Not Obvious over the Cited References

The claims of group VIII are directed to methods for replacing protein in a mammalian cell culture medium. The methods comprise replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate, wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

The claims of group VIII depend from claim 157 (which is included within group VII). Therefore, the methods encompassed by the claims of group VIII are not obvious over the references relied on in the rejection for at least the same reasons that the claim of group VII is not obvious over the references. *See* Section *VIII.B.7*, above. In addition, none of the references teach or suggest Fe^{2+} and/or Fe^{3+} at a concentration of about 0.00028 to 0.011 g/L, *and* Zn^{2+} at a concentration of about 0.00007 to 0.00073 g/L (claim 159); none of the references teach or suggest Fe^{2+} and/or Fe^{3+} at a concentration of about 0.011 g/L, *and* Zn^{2+} at a concentration of about 0.000354 g/L (claim 160). An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since not all elements of the claims of group VIII are taught or suggested by the references relied on in the rejection, the claims of group VIII are necessarily not obvious over the references.

9. The Claim of Group IX (Claim 161) is Not Obvious over the Cited References

The claim of group IX is directed to methods of cultivating 293 cells in suspension *in vitro*. The methods comprise obtaining 293 cells to be cultivated in suspension, and contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension. The claim of group IX is not obvious over the references relied on in the rejection.

As mentioned above, none of the cited references teach or suggest the suspension culture of mammalian cells. *See* Section *VIII.B.1*, above. There is no suggestion to combine or modify the cited references to achieve the presently claimed invention. *See id.* In addition, none of the references teach or suggest the use of 293 cells. *See* Section *VIII.B.5*, above. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since not all elements of the claim of group IX are taught or suggested by the references relied on in the rejection, the claim of group IX is necessarily not obvious over the references.

10. The Claims of Group X (Claims 162-174) are Not Obvious over the Cited References

The claims of group X are directed to methods of cultivating 293 cells in suspension *in vitro*. The methods comprise obtaining 293 cells to be cultivated in suspension, and contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension, and wherein the medium comprises at least one polyanionic or polycationic compound. The claims of group X are not obvious over the references relied on in the rejection.

The claims of group X depend directly or indirectly from claim 161 (which is included within group IX). Therefore, the methods encompassed by the claims of group X are not obvious over the references relied on in the rejection for at least the same reasons that the claim of group IX is not obvious over the references. *See* Section *VIII.B.9*, above. In addition, neither Chessebeuf nor Parenteau teach or suggest a culture medium comprising at least one polyanionic or polycationic compound, and Shuler teaches away from the use of sulfated polyanions in the culture of mammalian cells. *See* Section *VIII.B.1*, above. Since there is no suggestion to combine or modify the references relied on in the rejection, and since not all of the elements of the claims are taught or suggested by the cited references, the subject matter of the claims of group X is not obvious over the references.

C. Errors in the Rejection

1. Not All Elements of the Claims are Found in the Cited References And No Evidence Has Been Presented to Indicate a Motivation to Combine or Modify the References

In order to establish a *prima facie* case of obviousness, the Examiner must clearly demonstrate that: (a) all elements of the claims are taught or suggested by the cited references, and (b) there is a suggestion or motivation to modify or combine the reference teachings. *See* Section *VIII.A*, above. A *prima facie* case of obviousness has not been established with respect to the claims involved in this appeal because not all elements of the claims have been shown to be present in the cited references, and the Examiner has not presented any evidence to suggest that a person of ordinary skill in the art would have been motivated to combine or modify the references relied on in the rejection.

As the discussion set forth in Section VIII.B of this Brief demonstrates, there are several elements included within the claims that are not taught or suggested by the cited references. For example, none of the references teach or suggest:

1) obtaining a *mammalian cell* to be cultivated *in suspension*;

2) obtaining a 293 embryonic kidney cell (or a HeLa cervical epithelial cell, a PER-C6 retinal cell, or derivatives thereof) to be cultivated in suspension;

3) use of a 10X concentrated medium formulation;

4) contacting a mammalian cell with an adenovirus, an adeno-associated virus or a retrovirus;

5) replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate; and

6) culture media wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

The Examiner has not demonstrated that any of the above-listed elements are taught or suggested in the cited references. In fact, none of the elements listed above, except for elements 2 and 5 (use of a 293 cell and replacing insulin and transferrin, respectively), have even been addressed in the Office Actions. The Examiner's comments with respect to these elements are addressed in the discussion set forth below. Since the Examiner has not demonstrated that all of the elements of the claims are taught or suggested by the cited references, a *prima facie* case of obviousness has not been established.

In addition, the Examiner has not provided specific evidence to indicate that a person of ordinary skill in the art would have been motivated to modify or combine the references.

Several assertions have been put forth by the Examiner to support the rejection. These assertions are addressed individually below. In many cases, the Examiner's assertions in support of the rejection are simply conclusions that are not supported by any particular evidence. As noted by the Federal Circuit, the showing of combinability, in whatever form, must be "clear and particular." *Dembiczak*, 175 F.3d at 999. "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence.'" *Id.* Since no clear and particular evidence has been put forth to demonstrate a motivation to combine or modify the cited references, a *prima facie* case of obviousness has not been established.

2. The Examiner's Assertions In Support of the Rejection Are Legally Insufficient to Establish a Prima Facie Case of Obviousness

The Examiner's assertions in support of the rejection are addressed in turn below.

The Examiner stated that:

> It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to replace the dextran as disclosed by Chessebeuf with heparin or some other related and like compound to culture mammal cells as disclosed by Shuler et al.

Office Action dated July 30, 2002, page 5, lines 5-7. This statement does not support a *prima facie* case of obviousness. First, it is not supported by any evidence. The Examiner has not pointed to anything in particular to suggest replacing dextran in the medium of Chessebeuf with "heparin or some other related and like compound." Second, the replacement suggested by the Examiner would not result in a method that falls within the scope of any of the claims because Chessebeuf does not teach or suggest obtaining a mammalian cell to be cultivated *in suspension*.

With respect to the combination of Chessebeuf and Shuler, the Examiner made several additional assertions. First, the Examiner stated that "the references have been applied in combination, and any deficiency of Chessebeuf is remedied by the application of Shuler which does teach use of sulfated polyanions for cell culture." Office Action dated February 25, 2003, page 2, lines 18-20. It is undisputed that the Examiner has combined the references in making the rejection. A *prima facie* case of obviousness, however, cannot be established simply by combining references. There must be some suggestion or motivation to make the combination. Here, no specific suggestion or motivation to combine Chessebeuf and Shuler has been articulated by the Examiner. As discussed elsewhere in this Brief, Shuler only relates to *insect* cell culture.

The Examiner also stated:

> Albeit the invention of Shuler is drawn to insect cells, the background of Shuler does teach that dextran sulfate has produced problems for animal cells and mammal cells. The teach[ing] of animal cells encompasses insect cells as well does animal cells encompass mammalian cells. Thus, the teachings of Shuler do indeed suggest that polyanionic compounds will provide successful results for the culturing of mammal cells, as claimed herein and taught by Chessebeuf et al.

Office Action dated February 25, 2003, page 2, line 20, through page 3, line 5. This argument is inaccurate and logically flawed. First, it is unclear exactly which statements in the background of Shuler the Examiner is referring to. The statements in the background of Shuler are primarily concerned with distinguishing the subject matter of Shuler from the prior art. *See, e.g.*, Shuler at column 3, lines 40-47. Second, regardless of whether Shuler indicates that dextran sulfate has produced problems for animal cells and mammalian cells, such statements would not suggest replacing dextran sulfate with other sulfated polyanions,

especially since Shuler indicates that sulfated polyanions cause mammalian cell aggregation; a person of ordinary skill in the art would appreciate that effective cultivation of mammalian cells in suspension requires that the cells do not aggregate. Third, it is unclear how the Examiner's statement that mammalian cells and insect cells are animal cells relates to the issue of whether a person of ordinary skill in the art would have been motivated to combine Shuler and Chessebeuf. A person of ordinary skill in the art would recognize that, even though insect cells and mammalian cells are animal cells, the conditions and procedures associated with culturing insect cells are significantly different from those associated with culturing mammalian cells. These differences are made explicit by Shuler who notes that the behavior of mammalian cells in the presence of sulfated polyanions is the opposite of that which is observed with insect cells. Thus, the Examiner's statements quoted above do not support a *prima facie* case of obviousness.

The Examiner also stated:

> Shuler determined that at specific concentrations dextran sulfate can be an useful polyanion. Also note that insect cells are eucaryotic cell types and considered to be animal cells. Shuler does disclose that dextran sulfate only works at 25 ug/ml thus, one of ordinary skill in the art would have expected dextran sulfate and other polyanions to work and provide successful results for cultivation [of] animal cells in suspension.

Advisory Action dated October 14, 2003, page 2, lines 13-18. This statement does not support a *prima facie* case of obviousness. First, the demonstration by Shuler that dextran sulfate "only works at 25 ug/ml" does not suggest substituting the specific insect cell line used in the experiments of Shuler with mammalian cells. Second, as discussed above, the fact that both insect cells and mammalian cells are animal cells does not suggest that mammalian cells can be used with the culture medium of Shuler. The culture medium of

Shuler was developed for a specific insect cell line (TN5B1-4). There is no indication that the medium would be useful in culturing mammalian cells. More importantly, there is no *specific suggestion* to use the culture medium of Shuler to culture mammalian cells. Indeed, as discussed elsewhere in this Brief, Shuler teaches away from using sulfated polyanions to culture mammalian cells.

The Examiner also stated that "Shuler is teaching toward dextran sulfate as well as pentosan sulfate as being used for suspension culturing of animal cells. To culture other animal cells such as mammalian cells is clearly within the purview of an ordinary artisan." Advisory Action dated October 14, 2003, page 3, lines 1-4. This statement does not support a *prima facie* case of obviousness. A *prima facie* case of obviousness cannot be established on the basis of what is asserted to be "within the purview of an ordinary artisan." There must be a specific suggestion to combine references. *See In re Mills*, 916 F.2d 680,682 (Fed. Cir. 1992) (Although a prior art device "may be capable of being modified to run the way the apparatus is claimed, there must be a suggestion or motivation in the reference to do so.") *See also In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984) ("The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.") Whether or not something is "within the purview of an ordinary artisan" does not indicate a motivation to combine references.

With respect to the establishment of a *prima facie* case of obviousness, the Examiner stated that "[a]ll that is needed in order to establish a prima facie case of obviousness is some suggestion to combine the teachings. The examiner thinks that this suggestion is provided by the cited prior art combination." Office Action dated February 25, 2003, page

3, lines 5-7. This is an inaccurate assertion of the legal requirement for establishing a *prima facie* case of obviousness. Other elements, in addition to motivation, must be established in order to establish a *prima facie* case of obviousness. *See* Section *VIII.A*, above. Moreover, any asserted suggestion to combine references must be clearly articulated by the Examiner. *See Dembiczak*, 175 F.3d at 999. It is not sufficient to simply state that the suggestion "is provided by the cited prior art combination." More particular evidence of a motivation to combine must be provided.

As mentioned above, Shuler would be regarded by a person of ordinary skill in the art as teaching away from the use of sulfated polyanions in culturing mammalian cells. *See* Section *VIII.B.1*, above. There is no suggestion to combine references if a reference teaches away from the combination. *See Tec Air, Inc.* 192 F.3d at 1360. In response, the Examiner stated that "by no means is Shuler teaching away from using dextran sulfate but to the contrary is directing one of skill in the art to its use for suspension culturing to minimize aggregation by controlling its concentration." Advisory Action dated October 14, 2003, page 3, lines 6-8. This statement does not refute Appellants' contention that Shuler teaches away from the claimed invention. Appellants' assertion is not simply that Shuler teaches away from using dextran sulfate, but that Shuler teaches away from using sulfated polyanions generally in the culture of mammalian cells. In support of this assertion, Appellants have pointed to, *inter alia*, Shuler at column 12, lines 1-13. The Examiner stated that this section of Shuler "is based on other studies of the background art, and notably Nakashima et al, where it was reported that polyanions such as dextran sulfate induce aggregation of mouse lymphocytes." Advisory Action dated October 14, 2003, page 2, lines 11-13. The portion of Shuler cited by Appellants (column 12, lines 1-13) indicates that

sulfated polyanions induce aggregation of mammalian cells. The impact the statement would have on persons of ordinary skill in the art would not be diminished simply because the statement refers to the work of others. A person of ordinary skill in the art, upon reading Shuler in its entirety would conclude that the results obtained in Shuler are specific to insect cells and that opposite effects (*i.e.*, cell aggregation) would likely result if sulfated polyanions are used in the culture of mammalian cells.

As noted above, there are several elements of the claims that are not taught or suggested by the references relied on in the rejection. One such element is obtaining a 293 embryonic kidney cell to be cultivated in suspension. This element is found in claims 33, 34, 76 and 161-172 but is not found in any of the cited references. The Examiner apparently recognized this deficiency but nevertheless asserted that 293 cells "would not have been expected to be cultured any differently from other animal cell lines disclosed in the art and based on knowledge generally available to one of ordinary skill in the art." Office Action dated February 25, 2003, page 3, lines 9-10. The Examiner has not presented any evidence to support this position. As noted in Appellants' Reply filed on August 25, 2003, it appears that the Examiner has taken "official notice" of what would (or would not) have been expected in the art with respect to 293 cells. Such an attempt to take official notice is improper under the guidelines of MPEP § 2144.03. *See* Appellants' Reply filed on August 25, 2003, pages 4-7; *see also In re Lee*, 277 F.3d 1338, 1343-44 (Fed. Cir. 2002) ("This factual question of motivation is material to patentability, and could not be resolved on subjective belief and unknown authority.")

The Examiner responded by pointing to Appellants' specification at page 9, lines 1-20, where, according to the Examiner, "Applicants clearly disclose that these cells [*i.e.*, 293

cells] are well known in the art." Advisory Action dated October 14, 2003, page 3, lines 18-19. It is well established that the requisite motivation to modify a reference teaching cannot be derived from an Applicant's own disclosure. *See In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). Thus, the Examiner cannot properly rely on what is stated in Appellants' specification to show motivation to modify the references.

Notwithstanding the fact that the Examiner has not provided any valid evidence to support the assertion that 293 cells "would not have been expected to be cultured any differently from other animal cell lines disclosed in the art," Appellants note that a *prima facie* case of obviousness requires a showing that all of the claim elements are taught or suggested by the cited references. *See Royka*, 490 F.2d at 985. Simply asserting that one of ordinary skill in the art would not have expected 293 cells to be cultured "any differently from other animal cell lines disclosed in the art," cannot substitute for the Examiner's burden of showing that all of the elements of the claims are taught or suggested by the prior art. None of the references relied on in the rejection teach or suggest the use of 293 cells. The Examiner has not presented any arguments or assertions that 293 cells are taught or suggested by the cited references, much less that there would have been a motivation to modify any of the cited references by including 293 cells. Thus, a *prima facie* case of obviousness cannot be established for claims that include the element of obtaining a 293 embryonic kidney cell to be cultivated in suspension.

Another element of the claims that is not taught or suggested by the references relied on in the rejection is replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. This element is found in claims 157, 159 and 160, but is not

found in any of the cited references. With respect to this element, the Examiner asserted that:

> It should be noted that such chelator as zinc and iron are well known by those of skill in the art and protein is a well known contaminant of cell culture medium. Thus, to eliminate protein without a need for its presence in a cell culture medium and especially when it's [sic] presence is well known to be undesirable it would have been obvious to those of skill in the art to eliminate protein from the mammalian cell culture medium.
>
> Especially since the protein would serve no function to a method for culturing the cells in the cell culture medium from which the protein has been eliminated due to its undesirable effect and function. Therefore, the replacement of transferrin and insulin with chelator such as zinc and iron would have been expected to provide successful results.

Office Action dated February 25, 2003, page 3, line 12, through page 4, line 1. This assertion appears to be another instance in which the Examiner has improperly taken official notice of facts without providing any specific evidence to support the assertion. *See* Appellants' Reply filed on August 25, 2003, page 8; *see also In re Lee*, 277 F.3d 1338, 1343-44 (Fed. Cir. 2002) ("This factual question of motivation is material to patentability, and could not be resolved on subjective belief and unknown authority.") The Examiner's statements therefore cannot contribute to the establishment of a *prima facie* case of obviousness in the absence of any specific evidence to support them.

Moreover, the issue of whether a person of ordinary skill in the art would want to eliminate proteins in culture medium is tangential to the issue of whether the cited art teaches or suggests all of the elements of the claims. The claim element at issue is replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. The Examiner has not pointed to anything which specifically teaches or suggests replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate, as

required by the claims. Since not all of the elements of the claims is taught or suggested by the cited references, a *prima facie* case of obviousness cannot be established.

Finally, the Examiner stated that "Parenteau clearly recognizes using Zn additives in absence of insulin and clearly teaches using transferring [sic: transferrin] or ferrous ions." Advisory Action dated October 14, 2003, page 4, lines 9-10. This statement does not support a *prima facie* case of obviousness. First, it is unclear to which specific part of Parenteau the Examiner's statement refers. Second, using zinc additives in the absence of insulin and using transferrin or ferrous ions is not the same as replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate, as recited by the claims. None of the cited references teaches or suggests replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. Thus, a *prima facie* case of obviousness cannot be established for claims that include the element of replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate.

D. Summary

The claims on appeal are not obvious over the references relied on in the rejection. The Examiner has not established a *prima facie* case of obviousness. First, not all of the elements of the claims are taught or suggested by the cited references. Combining the references therefore would not result in subject matter that falls within the scope of the claims. Second, notwithstanding the fact that not all claim elements are taught or suggested by the references, the Examiner has not demonstrated that a person of ordinary skill in the art would have been motivated to combine the references. The Examiner has not pointed to anything specific in the references that would suggest their combination or modification.

In view of the foregoing remarks, Appellants respectfully request that the Board reverse the Examiner's 35 U.S.C. § 103 rejection of claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 and remand this application for issue.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Frank R. Cottingham
Attorney for Appellants
Registration No. 50,437

Date: DEC. 29, 2003

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

IX. Appendix (37 C.F.R. § 1.192(c)(9))

1. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a serum-free, chemically defined cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

2. The method of claim 1, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

3. The method of claim 2, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

6. The method of claim 1, wherein said medium is protein-free.

7. The method of claim 1, wherein said medium is a 1X medium formulation.

8. The method of claim 1, wherein said medium formulation is a 10X concentrated medium formulation.

9. The method of claim 1, wherein said medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, transferrin substitutes, insulin, and insulin substitutes.

10. The method of claim 9, wherein said medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

11. The method of claim 10, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

12. The method of claim 9, wherein said amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

13. The method of claim 9, wherein said vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}.

14. The method of claim 9, wherein said inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts.

15. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a chemically defined cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, at least one polyanionic or polycationic compound, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

16. The method of claim 15, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

17. The method of claim 16, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

20. The method of claim 15, wherein said medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

21. The method of claim 20, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

22. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a serum-free, chemically defined cell culture medium obtained by combining a basal medium with at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

23. The method of claim 22, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

24. The method of claim 23, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

27. The method of claim 22, wherein said basal medium is obtained by combining one or more ingredients selected from the group consisting of ethanolamine, D-glucose, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, one or more calcium salts, one or more iron salts, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is added in an amount which supports the cultivation of said cell in suspension.

28. The method of claim 22, wherein said medium is obtained by combining said basal medium and one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

29. The method of claim 28, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

30. The method of any one of claims 1, 15 or 22, wherein said mammalian cell is a mammalian epithelial cell.

31. The method of claim 30, wherein said mammalian epithelial cell is selected from the group consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell, a tracheal epithelial cell, a kidney epithelial cell and a retinal epithelial cell.

32. The method of claim 30, wherein said cell is a human cell.

33. The method of claim 32, wherein said human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof.

34. The method of claim 33, wherein said human cell is a 293 embryonic kidney cell.

35. The method of claim 30, wherein said cell is a normal cell.

36. The method of claim 30, wherein said cell is an abnormal cell.

37. The method of claim 36, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

73. A method of producing a virus comprising

(a) obtaining a mammalian cell to be infected with a virus;

(b) contacting said cell with a virus under conditions suitable to promote the infection of said cell by said virus; and

(c) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of said virus by said cell.

74. The method of claim 73, wherein said mammalian cell is an epithelial cell.

75. The method of claim 73, wherein said mammalian cell is a human cell.

76. The method of claim 75, wherein said human cell is a 293 embryonic kidney cell.

77. The method of claim 73, wherein said virus is an adenovirus, an adeno-associated virus or a retrovirus.

140. The method of claim 1, wherein said serum-free cell culture medium is free of animal-derived ingredients.

154. The method of claim 15, wherein said medium is serum-free.

157. A method for replacing protein in a mammalian cell culture medium, said method comprising

replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate.

158. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a serum-free, non-animal derived cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

159. The method of claim 157, wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

160. The method of claim 159, wherein the concentration of Fe^{2+} and/or Fe^{3+} is about 0.0011 g/L and the concentration of Zn^{2+} is about 0.000354 g/L.

161. A method of cultivating 293 cells in suspension *in vitro*, comprising:

(a) obtaining 293 cells to be cultivated in suspension; and

(b) contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension.

162. The method of claim 161, wherein the medium further comprises at least one polyanionic or polycationic compound.

163. The method of claim 162, wherein the polyanionic compound is a polysulfonated compound or a polysulfated compound.

164. The method of claim 163, wherein the polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

165. The method of claim 162, wherein the polysulfonated or polysulfated compound is dextran sulfate.

166. The method of claim 165, wherein the dextran sulfate has an average molecular weight of about 5,000 dalton.

167. The method of claim 162, wherein the medium is protein-free.

168. The method of claim 162, wherein the medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more

vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, one or more transferrin substitutes, insulin, and one or more insulin substitutes.

169. The method of claim 168, wherein the medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

170. The method of claim 169, wherein the one or more plant peptides are one or more rice peptides or one or more soy peptides.

171. The method of claim 168, wherein the amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

172. The method of claim 168, wherein the vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}.

173. The method of claim 168, wherein the inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts.

174. The method of claim 165, wherein the dextran sulfate is present in the medium in an amount effective to substantially prevent clumping.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

GORFIEN *et al.*

Appl. No. 09/028,514

Filed: February 23, 1998

For: **Serum-Free Mammalian Cell Culture Medium, and Uses Thereof**

Confirmation No.: 4800

Art Unit: 1651

Examiner: Ware, D.

Atty. Docket: 0942.4110002/RWE/FRC

Brief on Appeal Under 37 C.F.R. § 1.192

Mail Stop Appeal Brief - Patents

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

A Notice of Appeal from the final rejection of claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 was filed on August 25, 2003. Appellants hereby file this Appeal Brief in triplicate, together with the required brief filing fee.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

I. Real Party in Interest (37 C.F.R. § 1.192(c)(1))

The real party in interest in this appeal is Invitrogen Corporation.

II. Related Appeals and Interferences (37 C.F.R. § 1.192(c)(2))

Appellants' undersigned representative is not aware of any appeals or interferences related to the captioned application.

III. Status of Claims (37 C.F.R. § 1.192(c)(3))

The captioned application was filed on February 23, 1998. As originally filed, the application contained a total of 139 claims.

In a preliminary amendment filed on September 24, 1999, claims 38-72, 84-105 and 113-139 were cancelled, claim 106 was amended, and claims 140-153 were added.

In an amendment filed on July 28, 2000, claims 78, 83, 141 and 142 were cancelled, claims 1, 15, 22, 106, 112, 146, 152 and 153 were amended, and claims 154-158 were added.

In an amendment filed on May 7, 2001, claims 152, 153 and 157 were amended, and claims 159 and 160 were added.

In an amendment filed on February 1, 2002, claims 4, 5, 18, 19, 25, 26, 110 and 111 were cancelled, claims 1, 3, 15, 17, 22, 24, 106, 107, 109 and 158 were amended, and claims 161-174 were added.

In an amendment filed on November 26, 2002, claims 9, 10 and 168 were amended.

Claims 1-3, 6-17, 20-24, 27-37, 73-77, 79-82, 106-109, 112, 140 and 143-174 are pending in the application.

Claims 79-82, 106-109, 112, 143-153, 155 and 156 are withdrawn from consideration.

Claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 are now on appeal. A copy of the claims on appeal can be found in the attached Appendix.

IV. Status of Amendments (37 C.F.R. § 1.192(c)(4))

All amendments have been entered. No amendments have been filed subsequent to the issuance of the final Office Action dated February 25, 2003.

V. Summary of Invention (37 C.F.R. § 1.192(c)(5))

In many biological applications that involve culturing mammalian cells, it is advantageous to grow the cells in suspension rather than in monolayers. *See* Specification at page 10, line 23, through page 12, line 8. For example, suspension cultures, which grow in a three-dimensional space, generally produce much higher cell yields than cells grown in monolayer culture. *See* Specification at page 11, lines 1-9. In addition, it is often desirable to grow mammalian cells in media that is free of serum and/or other undefined components that might interfere with the growth of the cells or complicate the isolation of biological products produced by cells in culture. *See* Specification at page 4, line 9, through page 7, line 2. Accordingly, the present invention relates to methods for culturing mammalian cells in suspension in serum-free media.

Independent claim 1 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a serum-free, chemically defined cell culture

medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate. Independent claim 15 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a chemically defined cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, at least one polyanionic or polycationic compound, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts, wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate. Claim 154 depends from claim 15 and specifies that the medium is serum-free. Independent claim 22 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a serum-free, chemically defined cell culture medium obtained by combining a basal medium with at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not

contain dextran sulfate. Claim 27 is directed to the method of claim 22, wherein said basal medium is obtained by combining one or more ingredients selected from the group consisting of ethanolamine, D-glucose, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, one or more calcium salts, one or more iron salts, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts, wherein each ingredient is added in an amount which supports the cultivation of said cell in suspension. Independent claim 158 is directed to a method of cultivating a mammalian cell in suspension *in vitro*, comprising: (a) obtaining a mammalian cell to be cultivated in suspension; and (b) contacting said cell with a serum-free, non-animal derived cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate. Independent claim 161 is directed to a method of cultivating 293 cells in suspension *in vitro*, comprising: (a) obtaining 293 cells to be cultivated in suspension; and (b) contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension. Claim 162 depends from claim 161 and specifies that the medium further comprises at least one polyanionic or polycationic compound. Claim 140 depends from

claim 1 and specifies that the serum-free cell culture medium is free of animal derived ingredients. Support for claims 1, 15, 22, 27, 140, 154, 158, 161 and 162 can be found throughout the specification, for example, at page 13, line 12, through page 15, line 2.

Claim 165 depends from claim 162 and specifies that the polysulfonated or polysulfated compound is dextran sulfate. Claim 166 depends from claim 165 and specifies that the dextran sulfate has an average molecular weight of about 5,000 Dalton. Claim 174 depends from claim 165 and specifies that the dextran sulfate is present in the medium in an amount effective to substantially prevent clumping (cell aggregation). Support for claims 165, 166 and 174 can be found throughout the specification, for example, at page 13, lines 19-20 and at page 32, lines 22-25.

Claims 2, 16, 23 and 163 depend from claims 1, 15, 22, and 162, respectively, and specify that the polyanionic compound is a polysulfonated compound or a polysulfated compound. Claims 3, 17, 24 and 164 depend from claims 2, 16, 23 and 164, respectively, and specify that the polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan. Support for claims 2, 3, 16, 17, 23, 24, 163 and 164 can be found throughout the specification, for example, at page 13, lines 16-20.

Claims 6 and 167 depend from claims 1 and 162, respectively, and specify that the medium is protein-free. Claims 7 and 8 depend from claim 1 and specify that the medium is a 1X medium formulation and a 10X concentrated medium formulation, respectively. Support for claims 6, 7, 8 and 167 can be found throughout the specification, for example, at page 20, lines 5-6.

Claims 9 and 168 depend from claims 1 and 162, respectively, and specify that the medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, transferrin substitutes, insulin, and insulin substitutes. Support for claims 9 and 168 can be found throughout the specification, for example, at page 30, lines 2-5.

Claims 10, 20, 28, and 169 depend from claims 9, 15, 22 and 168, respectively, and specify that the medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides. Claims 11, 21, 29 and 170 depend from claims 10, 20, 28, and 169, respectively, and specify that the one or more plant peptides are one or more rice peptides or one or more soy peptides. Claims 12 and 171 depend from claims 9 and 168, respectively, and specify that the amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine. Claims 13 and 172 depend from claims 9 and 168, respectively, and specify that the vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}. Claims 14 and 173 depend from claims 9 and 168, respectively, and specify that the inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$,

Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts. Support for claims 10-14, 20, 21, 28, 29 and 169-173 can be found throughout the specification, for example, at page 14, lines 1-18.

Independent claim 73 is directed to a method of producing a virus comprising (a) obtaining a mammalian cell to be infected with a virus; (b) contacting said cell with a virus under conditions suitable to promote the infection of said cell by said virus; and (c) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of said virus by said cell. Claim 77 depends from claim 73 and specifies that the virus is an adenovirus, an adeno-associated virus or a retrovirus. Support for claims 73 and 77 can be found throughout the specification, for example, at page 18, lines 14-22.

Claim 30, which depends from claims 1, 15 or 22, and claim 74, which depends from claim 73, specify that the mammalian cell is a mammalian epithelial cell. Claim 31 depends from claim 30 and specifies that the mammalian epithelial cell is selected from the group consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell, a tracheal epithelial cell, a kidney epithelial cell and a retinal epithelial cell. Claims 32 and 75 depend from claims 30 and 73, respectively, and specify that the cell is a human cell. Claim 33 depends from claim 32 and specifies that the human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof. Claims 34 and 76 depend from claims 33 and 75, respectively, and specify that the human cell is a 293 embryonic kidney cell. Claim 35 depends from claim 30 and specifies that the cell is a normal cell. Claim 36 depends from claim 30 and specifies that the cell is an abnormal cell. Claim 37 depends from claim 36 and specifies that the abnormal cell is a transformed cell,

an established cell, or a cell derived from a diseased tissue sample. Support for claims 30-36 and 74-76 can be found throughout the specification, for example, at page 16, line 8, through page 17, line 10.

Independent claim 157 is directed to a method for replacing protein in a mammalian cell culture medium, said method comprising replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. Claim 159 depends from claim 157 and specifies that Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L. Claim 160 depends from claim 159 and specifies that the concentration of Fe^{2+} and/or Fe^{3+} is about 0.0011 g/L and the concentration of Zn^{2+} is about 0.000354 g/L. Support for claims 157, 159 and 160 can be found throughout the specification, for example, at page 40, line 3, through page 41, line 3.

VI. Issue on Appeal (37 C.F.R. § 1.192(c)(6))

The issue on appeal is whether claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 are unpatentable under 35 U.S.C. § 103(a) as being obvious over U.S. Patent No. 4,786,599 to Chessebeuf *et al.* ("Chessebeuf") in view of U.S. Patent No. 5,728,580 to Shuler *et al.* ("Shuler") and U.S. Patent No. 5,712,163 to Parenteau *et al.* ("Parenteau"). *See* Office Action dated February 25, 2003, page 2, lines 11-13.

VII. Grouping of Claims (37 C.F.R. § 1.192(c)(7))

For the purpose of this appeal, the pending claims do not stand or fall together. The claims are grouped as follows:

Group I: Claims 1-3, 6, 7, 9-17, 20-24, 27-32, 35-37, 140, 154 and 158;

Group II: Claim 8;

Group III: Claims 33 and 34;

Group IV: Claims 73-75;

Group V: Claim 76;

Group VI: Claim 77;

Group VII: Claim 157;

Group VIII Claims 159 and 160;

Group IX: Claim 161; and

Group X: Claims 162-174.

VIII. Argument (37 C.F.R. § 1.192(c)(8))

A. Legal Standard for Obviousness

In order to establish a *prima facie* case of obviousness, three requirements must be met. First, all the claim limitations must be taught or suggested by the prior art. *See In re Royka*, 490 F.2d 981, 984-85 (CCPA 1974); *see also In re Glaug*, 283 F.3d 1335, 1341-42 (Fed. Cir. 2002) (finding a claim not obvious because the prior art did not teach "spaced zones of adhesive" as recited in the claim); *In re Rijckaert*, 9 F.3d 1531, 1533 (Fed. Cir. 1993) (finding a claim not obvious because the prior art did not teach all claim limitations). Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *See In re Rouffet,* 149 F.3d 1350, 1357 (Fed. Cir. 1998). Third, there must be a reasonable expectation of success. *See In re Merck & Co.,*

Inc., 800 F.2d 1091 (Fed. Cir. 1986). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in Applicants' disclosure. *See In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991).

Evidence of a suggestion, teaching, or motivation to combine prior art references may flow, *inter alia*, from the references themselves, the knowledge of one of ordinary skill in the art, or from the nature of the problem to be solved. *See In re Dembiczak*, 175 F.3d 994, 999 (Fed. Cir. 1999). Although a reference need not expressly teach that the disclosure contained therein should be combined with another, *see Motorola, Inc. v. Interdigital Tech. Corp.*, 121 F.3d 1461, 1472 (Fed. Cir. 1997), the showing of combinability, in whatever form, must nevertheless be "clear and particular." *Dembiczak*, 175 F.3d at 999. "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence.'" *Id.* at 999; *see also In re Kotzab*, 217 F.3d 1365, 1371 (Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed.")

B. The Subject Matter of the Claims is Not Obvious Over the Cited References

1. The Claims of Group I (Claims 1-3, 6, 7, 9-17, 20-24, 27-32, 35-37, 140, 154 and 158) are Not Obvious over the Cited References

The claims of group I are directed to methods for cultivating a mammalian cell in suspension *in vitro*. The methods comprise obtaining a mammalian cell to be cultivated in suspension, and contacting the cell with a culture medium (*e.g.*, a serum-free and/or chemically defined cell culture medium) comprising at least one polyanionic or polycationic

compound. The media used in the methods of the group I claims do not contain dextran sulfate. The claims of group I are not obvious over the references relied on in the rejection.

Chessebeuf relates to a serum-free culture medium supplemented with certain fatty acids or their esters in the presence of a "lipophile biopolymer." *See* Chessebeuf at column 2, lines 27-34. Chessebeuf does not teach or suggest a culture medium comprising at least one polyanionic or polycationic compound. Chessebeuf does not teach or suggest culturing a *mammalian* cell in suspension.

Shuler relates to culturing a particular *insect* cell line, BTI-Tn-5B1-4 ("TN5B1-4"), in suspension culture in the presence of dextran sulfate or other sulfated polyanions. *See* Shuler at column 3, lines 34-39. Shuler does not teach or suggest the suspension culture of *mammalian* cells. In fact, Shuler teaches away from the use of sulfated polyanions in the culture of mammalian cells; according to Shuler, such compounds have been found to induce aggregation of *mammalian* cells. For example, Shuler states that "[i]n mammalian cells, the results [with sulfated polyanions] are not clear-cut. For example, heparin induces aggregation of lymphoid cells . . ., and polyanions such as dextran sulfate induce aggregation of lymphocyte cells." Shuler at column 4, lines 55-59 (internal citations omitted). Shuler also notes that:

> Other studies have shown that *sulfated polysaccharides induce aggregation of mammalian cells, indicating that these compounds have no intrinsic capacity to disaggregate cultures*, and in fact, disaggregation is an unexpected result. Thurn and Underhill found that heparin and dextran sulfate induced aggregation in cells of lymphoid origins but not in cell lines of fibroblastic origins. The ability of heparin to induce aggregation is closely related to the binding affinity of heparin to the cell surface. Conditions such as a shift in pH or ionic strength which lower the binding affinity of heparin increase aggregation. Nakashima

> et al. reported that *polyanions such as dextran sulfate induce aggregation of mouse lymphocytes*.

Shuler at column 12, lines 1-13 (emphasis added).

The overall impression conveyed by Shuler is that the ability of sulfated polyanions to prevent cell aggregation is specific for TN5B1-4 insect cells. Shuler suggests that TN5B1-4 cells disaggregate in the presence of sulfated polyanions because of the presence of specific adhesion receptors on the surface of the cells. *See* Shuler at column 12, line 64, through column 13, line 14. Shuler distinguishes TN5B1-4 cells, not only from mammalian cells, but even more particularly from other insect cell lines (*e.g.*, SF21 cells), in terms of their ability to be disaggregated by sulfated polyanions. According to Shuler, "[a]dhesion receptors present on the surface of TN5B1-4, but not SF21 cells *interact specifically* with substrate on lysed membrane fragments causing aggregation of TN5B1-4 cells. Sulfated polyanions competitively bind to the substrate on the membrane fragments thus preventing aggregation of TN5B1-4 cells." Shuler at column 12, line 66, through column 13, line 4 (emphasis added). A person of ordinary skill in the art would therefore not expect that results observed using TN5B1-4 cells would be observed using cell types other than TN5B1-4.

Moreover, as mentioned above, Shuler indicates that sulfated polyanions cause mammalian cells to aggregate. A person of ordinary skill in the art would appreciate that effective cultivation of mammalian cells in suspension requires that the cells do not clump or aggregate. "There is no suggestion to combine . . . if a reference teaches away from its combination with another source." *Tec Air, Inc. v. Denso Manufacturing Michigan Inc.*, 192 F.3d 1353 (Fed. Cir. 1999). Thus, in view of Shuler, a person of ordinary skill in the art would have been discouraged from using sulfated polyanions in mammalian cell culture.

Parenteau relates to chemically defined media that allow the culture of cells in the absence of feeder cells, serum, or "other components which may contribute undefined proteins to the media." *See* Parenteau at column 6, line 66, through column 7, line 2. The media of Parenteau are used to culture attached cells or cells on microcarriers. *See* Parenteau at column 6, lines 9-13 and lines 34-40. Parenteau does not teach or suggest a culture medium comprising at least one polyanionic or polycationic compound. Parenteau does not teach or suggest culturing a mammalian cell in suspension.

There is no suggestion to combine or modify the cited references. In particular, a person of ordinary skill in the art would have not been motivated to include at least one polyanionic or polycationic compound in the culture media of Chessebeuf or Parenteau and/or to adapt the culture media of these references to cultivate cells in suspension. In addition, a person of ordinary skill in the art would not have been motivated to apply teachings for the particular insect cell line used in Shuler to mammalian cells. As discussed above, a person of ordinary skill in the art would have been discouraged from using the culture media of Shuler in conjunction with mammalian cells because Shuler indicates that sulfated polyanions induce aggregation of mammalian cells.

In addition, a person of ordinary skill in the art would not have had a reasonable expectation of success in combining the references. In particular, a person of ordinary skill in the art would have appreciated that the conditions and media used to culture insect cells differ significantly from those used to culture mammalian cells. A person of ordinary skill in the art would not have expected that mammalian cells could have successfully been cultured in the insect culture medium of Shuler. Since there is no suggestion to combine or modify the references relied on to craft the rejection, and since there would have been no

reasonable expectation of success in combining or modifying the cited references, the subject matter of the claims of group I cannot be said to be obvious over the references.

2. The Claim of Group II (Claim 8) is Not Obvious over the Cited References

The claim of group II is directed to methods for cultivating a mammalian cell in suspension *in vitro*. The methods comprise obtaining a mammalian cell to be cultivated in suspension, and contacting the cell with a serum-free, chemically defined culture medium comprising at least one polyanionic or polycationic compound. The media used in the methods of the group II claim do not contain dextran sulfate. The medium used in the methods of the group II claim is a 10X concentrated medium formulation.

The claim of group II depends from claim 1 (which is included within group I). The claim of group II therefore is not obvious over the references relied on in the rejection for at least the same reasons that the claims of group I are not obvious over the references. *See* Section *VIII.B.1*, above. In addition, none of the references teach or suggest a 10X concentrated medium formulation. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since not all elements of the claim of group II are taught or suggested by the references relied on in the rejection, the claim of group II is necessarily not obvious over the references.

3. The Claims of Group III (Claims 33 and 34) are Not Obvious over the Cited References

The claims of group III are directed to methods for cultivating a mammalian cell in suspension *in vitro*. The methods comprise obtaining a mammalian cell to be cultivated in suspension, and contacting the cell with a culture medium (*e.g.*, a serum-free and/or chemically defined cell culture medium) comprising at least one polyanionic or polycationic compound. The media used in the methods of the group III claims do not contain dextran sulfate. The claims of group III specify that the mammalian cell is a human cell, wherein the human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof.

The claims of group III depend from claim 32 (which is included within group I). The claims of group III therefore are not obvious over the references relied on in the rejection for at least the same reasons that the claims of group I are not obvious over the references. *See* Section *VIII.B.1*, above. In addition, none of the references teach or suggest methods comprising culturing 293 embryonic kidney cells, HeLa cervical epithelial cells, PER-C6 retinal cells, or a derivatives thereof. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since the particular human cells specified in the claims of group III are not taught or suggested by the references relied on in the rejection, the claims of group III are necessarily not obvious over the references.

4. The Claims of Group IV (Claims 73-75) are Not Obvious over the Cited References

The claims of group IV are directed to methods for producing a virus. The methods comprise obtaining a mammalian cell to be infected with a virus; contacting the cell with a virus under conditions suitable to promote the infection of the cell by the virus; and cultivating the cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of the virus by the cell.

The methods encompassed by the claims of group IV comprise methods of cultivating a mammalian cell in suspension *in vitro* as defined by claims 1, 15, or 22 (which are included within group I). The claims of group IV therefore are not obvious over the references relied on in the rejection for at least the same reasons that the claims of group I are not obvious over the references. *See* Section *VIII.B.1*, above. In addition, none of the references teach or suggest methods of producing a virus comprising contacting a mammalian cell with a virus. Shuler mentions the infection of TN5B1-4 insect cells with a recombinant E2 strain of *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV). *See* Shuler at column 7, lines 22-32. There is no suggestion in Shuler of infecting mammalian cells with a virus. As mentioned above, Shuler teaches away from the use of mammalian cells in the context of the methods described therein. Accordingly, the claims of group IV are not obvious over the references relied on to craft the rejection.

5. The Claim of Group V (Claim 76) is Not Obvious over the Cited References

The claim of group V is directed to methods for producing a virus. The methods comprise obtaining a mammalian cell to be infected with a virus; contacting the cell with a

virus under conditions suitable to promote the infection of the cell by the virus; and cultivating the cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of the virus by the cell. The claim of group V specifies that the mammalian cell is a human 293 embryonic kidney cell.

The claim of group V depends from claim 75 (which is included within group IV). The methods encompassed by the claim of group V comprise methods of cultivating a mammalian cell in suspension *in vitro* as defined by claims 1, 15, or 22 (which are included within group I). Therefore, the methods encompassed by the claim of group V are not obvious over the references relied on in the rejection for at least the same reasons that the claims of groups I and IV are not obvious over the references. *See* Sections *VIII.B.1* and *4*, above. In addition, none of the references teach or suggest the use of human 293 embryonic kidney cells. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since human 293 embryonic kidney cells are not taught or suggested by the references relied on in the rejection, the claims of group V are necessarily not obvious over the references.

6. The Claim of Group VI (Claim 77) is Not Obvious over the Cited References

The claim of group VI is directed to methods for producing a virus. The methods comprise obtaining a mammalian cell to be infected with a virus; contacting the cell with a virus under conditions suitable to promote the infection of the cell by the virus; and cultivating the cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of the virus by the cell. The claim of group VI specifies that the virus is an adenovirus, an adeno-associated virus or a retrovirus.

The claim of group VI depends from claim 73 (which is included within group IV). The methods encompassed by the claim of group VI comprise methods of cultivating a mammalian cell in suspension *in vitro* as defined by claims 1, 15, or 22 (which are included within group I). Therefore, the methods encompassed by the claim of group VI are not obvious over the references relied on in the rejection for at least the same reasons that the claims of groups I and IV are not obvious over the references. *See* Sections *VIII.B.1* and *4*, above. In addition, none of the references teach or suggest the use of an adenovirus, an adeno-associated virus or a retrovirus. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since neither adenoviruses, adeno-associated viruses nor retroviruses are taught or suggested by the references relied on in the rejection, the claims of group VI are necessarily not obvious over the references.

7. The Claim of Group VII (Claim 157) is Not Obvious over the Cited References

The claim of group VII is directed to methods for replacing protein in a mammalian cell culture medium. The methods comprise replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. The claim of group VII is not obvious over the references relied on in the rejection.

None of the references teach or suggest the replacement of protein in a mammalian cell culture medium. More specifically, none of the references teach or suggest (i) replacing insulin with a Zn^{2+} salt; (ii) replacing transferrin with a Fe^{2+} chelate; and/or (iii) replacing transferrin with a Fe^{3+} chelate. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d

at 984-85. Since not all elements of the claim of group VII are taught or suggested by the references relied on in the rejection, the claim of group VII is not obvious over the references.

8. The Claims of Group VIII (Claims 159 and 160) are Not Obvious over the Cited References

The claims of group VIII are directed to methods for replacing protein in a mammalian cell culture medium. The methods comprise replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate, wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

The claims of group VIII depend from claim 157 (which is included within group VII). Therefore, the methods encompassed by the claims of group VIII are not obvious over the references relied on in the rejection for at least the same reasons that the claim of group VII is not obvious over the references. *See* Section *VIII.B.7*, above. In addition, none of the references teach or suggest Fe^{2+} and/or Fe^{3+} at a concentration of about 0.00028 to 0.011 g/L, *and* Zn^{2+} at a concentration of about 0.00007 to 0.00073 g/L (claim 159); none of the references teach or suggest Fe^{2+} and/or Fe^{3+} at a concentration of about 0.011 g/L, *and* Zn^{2+} at a concentration of about 0.000354 g/L (claim 160). An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since not all elements of the claims of group VIII are taught or suggested by the references relied on in the rejection, the claims of group VIII are necessarily not obvious over the references.

9. The Claim of Group IX (Claim 161) is Not Obvious over the Cited References

The claim of group IX is directed to methods of cultivating 293 cells in suspension *in vitro*. The methods comprise obtaining 293 cells to be cultivated in suspension, and contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension. The claim of group IX is not obvious over the references relied on in the rejection.

As mentioned above, none of the cited references teach or suggest the suspension culture of mammalian cells. *See* Section *VIII.B.1*, above. There is no suggestion to combine or modify the cited references to achieve the presently claimed invention. *See id.* In addition, none of the references teach or suggest the use of 293 cells. *See* Section *VIII.B.5*, above. An obviousness rejection requires, among other things, that all of the claim elements be taught or suggested by the cited references. *See Royka*, 490 F.2d at 984-85. Since not all elements of the claim of group IX are taught or suggested by the references relied on in the rejection, the claim of group IX is necessarily not obvious over the references.

10. The Claims of Group X (Claims 162-174) are Not Obvious over the Cited References

The claims of group X are directed to methods of cultivating 293 cells in suspension *in vitro*. The methods comprise obtaining 293 cells to be cultivated in suspension, and contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension, and wherein the medium comprises at least one polyanionic or polycationic compound. The claims of group X are not obvious over the references relied on in the rejection.

The claims of group X depend directly or indirectly from claim 161 (which is included within group IX). Therefore, the methods encompassed by the claims of group X are not obvious over the references relied on in the rejection for at least the same reasons that the claim of group IX is not obvious over the references. *See* Section *VIII.B.9*, above. In addition, neither Chessebeuf nor Parenteau teach or suggest a culture medium comprising at least one polyanionic or polycationic compound, and Shuler teaches away from the use of sulfated polyanions in the culture of mammalian cells. *See* Section *VIII.B.1*, above. Since there is no suggestion to combine or modify the references relied on in the rejection, and since not all of the elements of the claims are taught or suggested by the cited references, the subject matter of the claims of group X is not obvious over the references.

C. Errors in the Rejection

1. Not All Elements of the Claims are Found in the Cited References And No Evidence Has Been Presented to Indicate a Motivation to Combine or Modify the References

In order to establish a *prima facie* case of obviousness, the Examiner must clearly demonstrate that: (a) all elements of the claims are taught or suggested by the cited references, and (b) there is a suggestion or motivation to modify or combine the reference teachings. *See* Section *VIII.A*, above. A *prima facie* case of obviousness has not been established with respect to the claims involved in this appeal because not all elements of the claims have been shown to be present in the cited references, and the Examiner has not presented any evidence to suggest that a person of ordinary skill in the art would have been motivated to combine or modify the references relied on in the rejection.

As the discussion set forth in Section VIII.B of this Brief demonstrates, there are several elements included within the claims that are not taught or suggested by the cited references. For example, none of the references teach or suggest:

1) obtaining a *mammalian cell* to be cultivated *in suspension*;

2) obtaining a 293 embryonic kidney cell (or a HeLa cervical epithelial cell, a PER-C6 retinal cell, or derivatives thereof) to be cultivated in suspension;

3) use of a 10X concentrated medium formulation;

4) contacting a mammalian cell with an adenovirus, an adeno-associated virus or a retrovirus;

5) replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate; and

6) culture media wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

The Examiner has not demonstrated that any of the above-listed elements are taught or suggested in the cited references. In fact, none of the elements listed above, except for elements 2 and 5 (use of a 293 cell and replacing insulin and transferrin, respectively), have even been addressed in the Office Actions. The Examiner's comments with respect to these elements are addressed in the discussion set forth below. Since the Examiner has not demonstrated that all of the elements of the claims are taught or suggested by the cited references, a *prima facie* case of obviousness has not been established.

In addition, the Examiner has not provided specific evidence to indicate that a person of ordinary skill in the art would have been motivated to modify or combine the references.

Several assertions have been put forth by the Examiner to support the rejection. These assertions are addressed individually below. In many cases, the Examiner's assertions in support of the rejection are simply conclusions that are not supported by any particular evidence. As noted by the Federal Circuit, the showing of combinability, in whatever form, must be "clear and particular." *Dembiczak*, 175 F.3d at 999. "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not 'evidence.'" *Id.* Since no clear and particular evidence has been put forth to demonstrate a motivation to combine or modify the cited references, a *prima facie* case of obviousness has not been established.

2. The Examiner's Assertions In Support of the Rejection Are Legally Insufficient to Establish a Prima Facie Case of Obviousness

The Examiner's assertions in support of the rejection are addressed in turn below.

The Examiner stated that:

> It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to replace the dextran as disclosed by Chessebeuf with heparin or some other related and like compound to culture mammal cells as disclosed by Shuler et al.

Office Action dated July 30, 2002, page 5, lines 5-7. This statement does not support a *prima facie* case of obviousness. First, it is not supported by any evidence. The Examiner has not pointed to anything in particular to suggest replacing dextran in the medium of Chessebeuf with "heparin or some other related and like compound." Second, the replacement suggested by the Examiner would not result in a method that falls within the scope of any of the claims because Chessebeuf does not teach or suggest obtaining a mammalian cell to be cultivated *in suspension*.

With respect to the combination of Chessebeuf and Shuler, the Examiner made several additional assertions. First, the Examiner stated that "the references have been applied in combination, and any deficiency of Chessebeuf is remedied by the application of Shuler which does teach use of sulfated polyanions for cell culture." Office Action dated February 25, 2003, page 2, lines 18-20. It is undisputed that the Examiner has combined the references in making the rejection. A *prima facie* case of obviousness, however, cannot be established simply by combining references. There must be some suggestion or motivation to make the combination. Here, no specific suggestion or motivation to combine Chessebeuf and Shuler has been articulated by the Examiner. As discussed elsewhere in this Brief, Shuler only relates to *insect* cell culture.

The Examiner also stated:

> Albeit the invention of Shuler is drawn to insect cells, the background of Shuler does teach that dextran sulfate has produced problems for animal cells and mammal cells. The teach[ing] of animal cells encompasses insect cells as well does animal cells encompass mammalian cells. Thus, the teachings of Shuler do indeed suggest that polyanionic compounds will provide successful results for the culturing of mammal cells, as claimed herein and taught by Chessebeuf et al.

Office Action dated February 25, 2003, page 2, line 20, through page 3, line 5. This argument is inaccurate and logically flawed. First, it is unclear exactly which statements in the background of Shuler the Examiner is referring to. The statements in the background of Shuler are primarily concerned with distinguishing the subject matter of Shuler from the prior art. *See, e.g.*, Shuler at column 3, lines 40-47. Second, regardless of whether Shuler indicates that dextran sulfate has produced problems for animal cells and mammalian cells, such statements would not suggest replacing dextran sulfate with other sulfated polyanions,

especially since Shuler indicates that sulfated polyanions cause mammalian cell aggregation; a person of ordinary skill in the art would appreciate that effective cultivation of mammalian cells in suspension requires that the cells do not aggregate. Third, it is unclear how the Examiner's statement that mammalian cells and insect cells are animal cells relates to the issue of whether a person of ordinary skill in the art would have been motivated to combine Shuler and Chessebeuf. A person of ordinary skill in the art would recognize that, even though insect cells and mammalian cells are animal cells, the conditions and procedures associated with culturing insect cells are significantly different from those associated with culturing mammalian cells. These differences are made explicit by Shuler who notes that the behavior of mammalian cells in the presence of sulfated polyanions is the opposite of that which is observed with insect cells. Thus, the Examiner's statements quoted above do not support a *prima facie* case of obviousness.

The Examiner also stated:

> Shuler determined that at specific concentrations dextran sulfate can be an useful polyanion. Also note that insect cells are eucaryotic cell types and considered to be animal cells. Shuler does disclose that dextran sulfate only works at 25 ug/ml thus, one of ordinary skill in the art would have expected dextran sulfate and other polyanions to work and provide successful results for cultivation [of] animal cells in suspension.

Advisory Action dated October 14, 2003, page 2, lines 13-18. This statement does not support a *prima facie* case of obviousness. First, the demonstration by Shuler that dextran sulfate "only works at 25 ug/ml" does not suggest substituting the specific insect cell line used in the experiments of Shuler with mammalian cells. Second, as discussed above, the fact that both insect cells and mammalian cells are animal cells does not suggest that mammalian cells can be used with the culture medium of Shuler. The culture medium of

Shuler was developed for a specific insect cell line (TN5B1-4). There is no indication that the medium would be useful in culturing mammalian cells. More importantly, there is no *specific suggestion* to use the culture medium of Shuler to culture mammalian cells. Indeed, as discussed elsewhere in this Brief, Shuler teaches away from using sulfated polyanions to culture mammalian cells.

The Examiner also stated that "Shuler is teaching toward dextran sulfate as well as pentosan sulfate as being used for suspension culturing of animal cells. To culture other animal cells such as mammalian cells is clearly within the purview of an ordinary artisan." Advisory Action dated October 14, 2003, page 3, lines 1-4. This statement does not support a *prima facie* case of obviousness. A *prima facie* case of obviousness cannot be established on the basis of what is asserted to be "within the purview of an ordinary artisan." There must be a specific suggestion to combine references. *See In re Mills*, 916 F.2d 680,682 (Fed. Cir. 1992) (Although a prior art device "may be capable of being modified to run the way the apparatus is claimed, there must be a suggestion or motivation in the reference to do so.") *See also In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984) ("The mere fact that the prior art could be so modified would not have made the modification obvious unless the prior art suggested the desirability of the modification.") Whether or not something is "within the purview of an ordinary artisan" does not indicate a motivation to combine references.

With respect to the establishment of a *prima facie* case of obviousness, the Examiner stated that "[a]ll that is needed in order to establish a prima facie case of obviousness is some suggestion to combine the teachings. The examiner thinks that this suggestion is provided by the cited prior art combination." Office Action dated February 25, 2003, page

3, lines 5-7. This is an inaccurate assertion of the legal requirement for establishing a *prima facie* case of obviousness. Other elements, in addition to motivation, must be established in order to establish a *prima facie* case of obviousness. *See* Section *VIII.A*, above. Moreover, any asserted suggestion to combine references must be clearly articulated by the Examiner. *See Dembiczak*, 175 F.3d at 999. It is not sufficient to simply state that the suggestion "is provided by the cited prior art combination." More particular evidence of a motivation to combine must be provided.

As mentioned above, Shuler would be regarded by a person of ordinary skill in the art as teaching away from the use of sulfated polyanions in culturing mammalian cells. *See* Section *VIII.B.1*, above. There is no suggestion to combine references if a reference teaches away from the combination. *See Tec Air, Inc.* 192 F.3d at 1360. In response, the Examiner stated that "by no means is Shuler teaching away from using dextran sulfate but to the contrary is directing one of skill in the art to its use for suspension culturing to minimize aggregation by controlling its concentration." Advisory Action dated October 14, 2003, page 3, lines 6-8. This statement does not refute Appellants' contention that Shuler teaches away from the claimed invention. Appellants' assertion is not simply that Shuler teaches away from using dextran sulfate, but that Shuler teaches away from using sulfated polyanions generally in the culture of mammalian cells. In support of this assertion, Appellants have pointed to, *inter alia*, Shuler at column 12, lines 1-13. The Examiner stated that this section of Shuler "is based on other studies of the background art, and notably Nakashima et al, where it was reported that polyanions such as dextran sulfate induce aggregation of mouse lymphocytes." Advisory Action dated October 14, 2003, page 2, lines 11-13. The portion of Shuler cited by Appellants (column 12, lines 1-13) indicates that

sulfated polyanions induce aggregation of mammalian cells. The impact the statement would have on persons of ordinary skill in the art would not be diminished simply because the statement refers to the work of others. A person of ordinary skill in the art, upon reading Shuler in its entirety would conclude that the results obtained in Shuler are specific to insect cells and that opposite effects (*i.e.*, cell aggregation) would likely result if sulfated polyanions are used in the culture of mammalian cells.

As noted above, there are several elements of the claims that are not taught or suggested by the references relied on in the rejection. One such element is obtaining a 293 embryonic kidney cell to be cultivated in suspension. This element is found in claims 33, 34, 76 and 161-172 but is not found in any of the cited references. The Examiner apparently recognized this deficiency but nevertheless asserted that 293 cells "would not have been expected to be cultured any differently from other animal cell lines disclosed in the art and based on knowledge generally available to one of ordinary skill in the art." Office Action dated February 25, 2003, page 3, lines 9-10. The Examiner has not presented any evidence to support this position. As noted in Appellants' Reply filed on August 25, 2003, it appears that the Examiner has taken "official notice" of what would (or would not) have been expected in the art with respect to 293 cells. Such an attempt to take official notice is improper under the guidelines of MPEP § 2144.03. *See* Appellants' Reply filed on August 25, 2003, pages 4-7; *see also In re Lee*, 277 F.3d 1338, 1343-44 (Fed. Cir. 2002) ("This factual question of motivation is material to patentability, and could not be resolved on subjective belief and unknown authority.")

The Examiner responded by pointing to Appellants' specification at page 9, lines 1-20, where, according to the Examiner, "Applicants clearly disclose that these cells [*i.e.*, 293

cells] are well known in the art." Advisory Action dated October 14, 2003, page 3, lines 18-19. It is well established that the requisite motivation to modify a reference teaching cannot be derived from an Applicant's own disclosure. *See In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). Thus, the Examiner cannot properly rely on what is stated in Appellants' specification to show motivation to modify the references.

Notwithstanding the fact that the Examiner has not provided any valid evidence to support the assertion that 293 cells "would not have been expected to be cultured any differently from other animal cell lines disclosed in the art," Appellants note that a *prima facie* case of obviousness requires a showing that all of the claim elements are taught or suggested by the cited references. *See Royka*, 490 F.2d at 985. Simply asserting that one of ordinary skill in the art would not have expected 293 cells to be cultured "any differently from other animal cell lines disclosed in the art," cannot substitute for the Examiner's burden of showing that all of the elements of the claims are taught or suggested by the prior art. None of the references relied on in the rejection teach or suggest the use of 293 cells. The Examiner has not presented any arguments or assertions that 293 cells are taught or suggested by the cited references, much less that there would have been a motivation to modify any of the cited references by including 293 cells. Thus, a *prima facie* case of obviousness cannot be established for claims that include the element of obtaining a 293 embryonic kidney cell to be cultivated in suspension.

Another element of the claims that is not taught or suggested by the references relied on in the rejection is replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. This element is found in claims 157, 159 and 160, but is not

found in any of the cited references. With respect to this element, the Examiner asserted that:

> It should be noted that such chelator as zinc and iron are well known by those of skill in the art and protein is a well known contaminant of cell culture medium. Thus, to eliminate protein without a need for its presence in a cell culture medium and especially when it's [sic] presence is well known to be undesirable it would have been obvious to those of skill in the art to eliminate protein from the mammalian cell culture medium.
>
> Especially since the protein would serve no function to a method for culturing the cells in the cell culture medium from which the protein has been eliminated due to its undesirable effect and function. Therefore, the replacement of transferrin and insulin with chelator such as zinc and iron would have been expected to provide successful results.

Office Action dated February 25, 2003, page 3, line 12, through page 4, line 1. This assertion appears to be another instance in which the Examiner has improperly taken official notice of facts without providing any specific evidence to support the assertion. *See* Appellants' Reply filed on August 25, 2003, page 8; *see also In re Lee*, 277 F.3d 1338, 1343-44 (Fed. Cir. 2002) ("This factual question of motivation is material to patentability, and could not be resolved on subjective belief and unknown authority.") The Examiner's statements therefore cannot contribute to the establishment of a *prima facie* case of obviousness in the absence of any specific evidence to support them.

Moreover, the issue of whether a person of ordinary skill in the art would want to eliminate proteins in culture medium is tangential to the issue of whether the cited art teaches or suggests all of the elements of the claims. The claim element at issue is replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. The Examiner has not pointed to anything which specifically teaches or suggests replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate, as

required by the claims. Since not all of the elements of the claims is taught or suggested by the cited references, a *prima facie* case of obviousness cannot be established.

Finally, the Examiner stated that "Parenteau clearly recognizes using Zn additives in absence of insulin and clearly teaches using transferring [sic: transferrin] or ferrous ions." Advisory Action dated October 14, 2003, page 4, lines 9-10. This statement does not support a *prima facie* case of obviousness. First, it is unclear to which specific part of Parenteau the Examiner's statement refers. Second, using zinc additives in the absence of insulin and using transferrin or ferrous ions is not the same as replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate, as recited by the claims. None of the cited references teaches or suggests replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate. Thus, a *prima facie* case of obviousness cannot be established for claims that include the element of replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate.

D. Summary

The claims on appeal are not obvious over the references relied on in the rejection. The Examiner has not established a *prima facie* case of obviousness. First, not all of the elements of the claims are taught or suggested by the cited references. Combining the references therefore would not result in subject matter that falls within the scope of the claims. Second, notwithstanding the fact that not all claim elements are taught or suggested by the references, the Examiner has not demonstrated that a person of ordinary skill in the art would have been motivated to combine the references. The Examiner has not pointed to anything specific in the references that would suggest their combination or modification.

In view of the foregoing remarks, Appellants respectfully request that the Board reverse the Examiner's 35 U.S.C. § 103 rejection of claims 1-3, 6-17, 20-24, 27-37, 73-77, 140, 154 and 157-174 and remand this application for issue.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Frank R. Cottingham
Attorney for Appellants
Registration No. 50,437

Date: DEC. 29, 2003

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

IX. Appendix (37 C.F.R. § 1.192(c)(9))

1. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a serum-free, chemically defined cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

2. The method of claim 1, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

3. The method of claim 2, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

6. The method of claim 1, wherein said medium is protein-free.

7. The method of claim 1, wherein said medium is a 1X medium formulation.

8. The method of claim 1, wherein said medium formulation is a 10X concentrated medium formulation.

9. The method of claim 1, wherein said medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, transferrin substitutes, insulin, and insulin substitutes.

10. The method of claim 9, wherein said medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

11. The method of claim 10, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

12. The method of claim 9, wherein said amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

13. The method of claim 9, wherein said vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}.

14. The method of claim 9, wherein said inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts.

15. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a chemically defined cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, at least one polyanionic or polycationic compound, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

16. The method of claim 15, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

17. The method of claim 16, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

20. The method of claim 15, wherein said medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

21. The method of claim 20, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

22. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a serum-free, chemically defined cell culture medium obtained by combining a basal medium with at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

23. The method of claim 22, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

24. The method of claim 23, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

27. The method of claim 22, wherein said basal medium is obtained by combining one or more ingredients selected from the group consisting of ethanolamine, D-glucose, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, one or more calcium salts, one or more iron salts, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is added in an amount which supports the cultivation of said cell in suspension.

28. The method of claim 22, wherein said medium is obtained by combining said basal medium and one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

29. The method of claim 28, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

30. The method of any one of claims 1, 15 or 22, wherein said mammalian cell is a mammalian epithelial cell.

31. The method of claim 30, wherein said mammalian epithelial cell is selected from the group consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell, a tracheal epithelial cell, a kidney epithelial cell and a retinal epithelial cell.

32. The method of claim 30, wherein said cell is a human cell.

33. The method of claim 32, wherein said human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof.

34. The method of claim 33, wherein said human cell is a 293 embryonic kidney cell.

35. The method of claim 30, wherein said cell is a normal cell.

36. The method of claim 30, wherein said cell is an abnormal cell.

37. The method of claim 36, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

73. A method of producing a virus comprising

(a) obtaining a mammalian cell to be infected with a virus;

(b) contacting said cell with a virus under conditions suitable to promote the infection of said cell by said virus; and

(c) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of said virus by said cell.

74. The method of claim 73, wherein said mammalian cell is an epithelial cell.

75. The method of claim 73, wherein said mammalian cell is a human cell.

76. The method of claim 75, wherein said human cell is a 293 embryonic kidney cell.

77. The method of claim 73, wherein said virus is an adenovirus, an adeno-associated virus or a retrovirus.

140. The method of claim 1, wherein said serum-free cell culture medium is free of animal-derived ingredients.

154. The method of claim 15, wherein said medium is serum-free.

157. A method for replacing protein in a mammalian cell culture medium, said method comprising

replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate.

158. A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; and

(b) contacting said cell with a serum-free, non-animal derived cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate.

159. The method of claim 157, wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

160. The method of claim 159, wherein the concentration of Fe^{2+} and/or Fe^{3+} is about 0.0011 g/L and the concentration of Zn^{2+} is about 0.000354 g/L.

161. A method of cultivating 293 cells in suspension *in vitro*, comprising:

(a) obtaining 293 cells to be cultivated in suspension; and

(b) contacting the cells with a serum-free, chemically defined cell culture medium,

wherein the medium supports the cultivation of the cell in suspension.

162. The method of claim 161, wherein the medium further comprises at least one polyanionic or polycationic compound.

163. The method of claim 162, wherein the polyanionic compound is a polysulfonated compound or a polysulfated compound.

164. The method of claim 163, wherein the polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

165. The method of claim 162, wherein the polysulfonated or polysulfated compound is dextran sulfate.

166. The method of claim 165, wherein the dextran sulfate has an average molecular weight of about 5,000 dalton.

167. The method of claim 162, wherein the medium is protein-free.

168. The method of claim 162, wherein the medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more

vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, one or more transferrin substitutes, insulin, and one or more insulin substitutes.

169. The method of claim 168, wherein the medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

170. The method of claim 169, wherein the one or more plant peptides are one or more rice peptides or one or more soy peptides.

171. The method of claim 168, wherein the amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

172. The method of claim 168, wherein the vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}.

173. The method of claim 168, wherein the inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts.

174. The method of claim 165, wherein the dextran sulfate is present in the medium in an amount effective to substantially prevent clumping.